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Characterization of acid hydrolysis of sisal

Clebson S.S. Lima [a], Marta M. Conceição [b,*], Flávio L.H. Silva [c], Ezenildo E. Lima [d], Líbia S. Conrado [d], Douglas A.S. Leão [d]

[a] *Department of Chemistry, CES, UFCG, Cuité, PB, Brazil*
[b] *Department of Chemistry, UAE, CES, UFCG, Rua Olho D'água da Bica, S/N Centro, Cuité, PB, CEP 58175000, Brazil*
[c] *Department of Process Engineering, CT, UFPB, João Pessoa, PB, Brazil*
[d] *Department of Process Engineering, UFCG, CCT, Campina Grande, PB, Brazil*

HIGHLIGHTS

- Pre hydrolysis at 105 °C was effective.
- Significant portion of xylose and arabinose was removed.
- The main product formed by acid hydrolysis at 160 °C was glucose.
- Ethanol was produced with 92% conversion of initial total reducing sugars.
- Sisal is a material that demonstrates feasibility for ethanol production.

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ABSTRACT

The present study aimed to characterize and evaluate the hydrolysis of sisal fibers for ethanol production. Acid hydrolysis of the fiber was performed and main products determined were xylose and glucose. However, xylose and arabinose (pentoses) are not fermented by the conventional yeast *Saccharomyces cerevisiae*. Prehydrolysis at 105 °C was efficient, removing most of the pentoses. The main product formed in acid hydrolysis at 160 °C was glucose, which can be metabolized by the yeast *S. cerevisiae* through alcoholic fermentation. In these conditions ethanol was produced with 92% conversion of initial total reducing sugars (0.47 g/g of ethanol yield). Sisal has potential as a raw material for ethanol production since it is not a food source, has high cellulose content and low biomass cost.

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1. Introduction

The population growth, higher vehicle numbers and atmospheric pollution caused by fossil fuels indicate the need for clean and renewable energy sources. Among the most widely studied new alternatives are biodiesel and bioethanol [1].

New technologies for producing second-generation bioethanol have enabled the use of agroindustrial wastes in bioethanol production; that is, converting the cellulose in this waste into fermentable sugars through acid or enzymatic hydrolysis [2].

Sugar as a renewable resource, can be derived from a variety of biological feedstocks such as lignocellulosic biomass. The sugar derived from lignocellulosic biomass can be further converted to a number of high-value bio-based fuels. The selection of the appropriate feedstock for biochemical production strongly depends on its local availability and cost. Currently, the most widely used feedstocks in tropical and sub-tropical regions, are from sugar cane; while in temperate regions, starchy products from crops such as corn, sorghum, wheat, rye, barley and potatoes are being used.

However, there is a drawback in producing biofuel and biochemical from sugar containing material or starch since these feedstocks are at the same time used as human food source and tends to be expensive and may cause the shortage of food supply. Therefore, recent interest is focused on the application of the low cost and abundantly available lignocellulosic biomass as potential feedstock for biofuel and biochemical production [3,4].

Lignocellulosic wastes, such as sisal and sugar cane bagasse, are being increasingly studied owing to their chemical composition, consisting mainly of cellulose, hemicellulose and lignin [2].

Originally from Mexico, the sisal plant (*Agave sisalana*) is cultivated in developing countries. In Brazil, plantations are concentrated in the states of Paraiba and Bahia. Sisal cultivation in the country has a planted area of around 154,000 ha, producing almost 800 kg/ha [5].

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[*] Corresponding author. Tel.: +55 8333721900; fax: +55 8333721945.
E-mail address: martamaria@ufcg.edu.br (M.M. Conceição).

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Sisal is a potential raw material for ethanol production given its relatively low biomass cost, the fact that it is not a food source and its high cellulose content, about 10% higher than sugar cane bagasse. In regard to hemicellulose, 90% of sisal fiber is material that generates fermentable sugars, which form ethanol through fermentation. These sugars are produced by hydrolysis, a reaction where bonds linking several glucose units in cellulose and other sugars in hemicellulose are broken [6,7].

In order to be employed as raw material, cellulose must be exposed to a catalyzer. To that end, lignocellulosic material must undergo pretreatment to completely or partially remove lignin and hemicellulose. Its compact and complex structure requires physical and/or chemical pre-treatment of the biomass before hydrolysis for ethanol production. Pre-treatment aims to remove lignin and hemicellulose, reduce cellulose crystallinity and increase porosity in order to make cellulose susceptible to hydrolysis. Regardless of the type of hydrolysis used, pretreatment is essential and several methods can be applied, including physical, chemical and biological [8].

Among all the pretreatment methods, dilute sulfuric acid pretreatment has been extensively studied because it is generally inexpensive, convenient and effective for a broad spectrum of lignocellulosic biomass (herbaceous, softwood, and hardwood) [9]. The dilute sulfuric acid pretreatment can effectively hydrolyze hemicellulose into monomeric sugars (arabinose, galactose, glucose, mannose, xylose, etc.) and soluble oligomers, thus improving cellulose conversion [10].

Pentosans (C5), namely xylose and arabinose, are more susceptible to thermal degradation than hexosans (C6), namely glucose, galactose, and mannose. Therefore, especially in those substrates containing significant amounts of C5, the pretreatment process should produce an effective biomass destructuration without compromising the recovery of C5 [11].

Fermentation is the phase where sugar-rich liquid from hydrolysis (hydrolyzed liquor) is fermented by the yeast *Saccharomyces cerevisiae*, the same fungus used to bake bread. In the final stage liquids from different fermentations are distilled. The product of this distillation is ethanol, which exhibits the same characteristics as those produced from sugar cane in industrial processes [12].

Brazil is the world's largest producer of sisal, which has a rapid growth cycle and high cellulose content. As such, it displays potential as raw material for the production of ethanol.

This work aims to realize preliminary characterization and evaluate the hydrolysis process of sisal fiber for ethanol production.

2. Materials and methods

Sisal samples (*A. sisalana*) were provided by a farmer from the municipality of Nova Floresta, in the state of Paraiba (Fig. 1).

Fig. 1. Sisal.

Sisal fiber was dried to constant weight at 75 °C in a circulating air oven and ground in a Tecnal Marconi TE340 knife mill.

2.1. Chemical composition of sisal

2.1.1. Moisture content

Fresh sisal samples in triplicate were placed in an oven at 105 °C. Five grams of sample was added to each crucible and kept in the oven for 24 h. Samples were then removed, cooled in a desiccator and weighed [13]. Moisture content was determined using the following equation:

$$Moisture\% = \frac{(initial\ weight\ \ - final\ sample\ weight)}{final\ sample\ weight} \times 100 \qquad (1)$$

2.1.2. Ash content

Total ash content was measured by calcination. Two grams of dry material was placed in a muffle furnace at 700 °C for 12 h and weighed after cooling in a desiccator [14].

2.1.3. Extractive content

Thirty grams of sample was submitted to extraction in a Soxhlet extractor for 6 h. Solvents used were n-hexane and ethanol at a ratio of 1:1 [15].

2.1.4. Lignin content

Lignin content was determined using the Klason method with insoluble lignin in 75% sulfuric acid. Samples were treated with 75% sulfuric acid and submitted to agitation for 2 h. The system was then heated to boiling point, followed by the addition of 560 mL of distilled water, diluting the acid solution to 3%. Lignin content was measured after filtration and drying [15].

2.1.5. Holocellulose

For the determination of holocellulose content was added 5 g sample in erlenmeyer, 0.75 g of sodium chlorite, 0.5 mL of concentrated acetic acid and 100 mL of distilled water, and the mixture kept under stirring until the chlorite was dissolved. The mixture was stirred at 75 °C for 1 h. The same amount of reagent was added every hour for a period of 3 h. The system was cooled with ice to prevent oxidation, and then filtered by vacuum filtration. The residue was washed with distilled water and ethanol, dried at 60 °C to constant mass and weighed. The holocellulose content was determined by the difference between initial and final mass [15].

2.1.6. Cellulose and hemicellulose

Chemical methods of separation of cellulose and hemicellulose were based on the methodology for sugarcane bagasse proposed by Xu et al. 2006 [16]. Adapted for sisal, 2 g sample was added 4.1 mL of distilled water, 14.5 mL of acetic acid and 1.37 mL of nitric acid. The samples were digested for 20 min at 60 °C under stirring.

After this time was added 200 mL of distilled water and the solution was digested for 40 min to separate the particles and facilitate filtration. After cooling, the samples were vacuum filtered and the precipitate was determined gravimetrically as cellulose total.

The alpha cellulose was determined by transferring the residue remaining from the previous procedure to a 250 mL erlenmeyer then added 100 mL of sodium hydroxide at 17.5% m/v and the mixture kept under stirring at room temperature, for 30 min.

After this time was added 100 mL of distilled water and remained 30 min of settling. The material was vacuum filtered with a quantitative filter paper, the filtrate was set aside for determination of beta cellulose, and the residue was washed with distilled water, dried at 60 °C for 24 h and weighed to quantify the alpha cellulose.

For determination of the beta cellulose in the filtrate was added 200 mL of 3N sulfuric acid (v/v). The solution was kept at rest for 24 h at a temperature of 7 °C took place for precipitation of beta cellulose. After this period the samples were filtered and vacuum dried at 60 °C for 24 h and determined by the gravimetric method.

The cellulose content was obtained by the sum of alpha and beta cellulose and hemicellulose content was obtained from the difference between holocellulose and cellulose.

2.2. Prehydrolysis (pretreatment)

The prehydrolysis was performed at 90 and 105 °C using 50 g of fiber in steel inox pressure reactor, 500 mL of 3% H_2SO_4. After prehydrolysis liquor was filtered in a vacuum system and the residue was washed with distilled water and dried at 60 °C to constant mass.

2.3. Acid Hydrolysis

The hydrolysis monitoring of sisal fiber was achieved using 3% H_2SO_4 in a ratio of 1:10 for fiber acid to the reaction time of 120 min, in order to evaluate the best time of extraction of glucose.

Hydrolysis of sisal fiber was conducted in a pressurized reactor using 3% sulfuric acid as a catalyzer at a fiber/acid ratio of 1/10 in the temperature of 160 °C for 1 h.

2.4. Adsorption of inhibitors using residual lignin

Adsorption was performed in hydrolyzed liquor at 160 °C. The liquor underwent adsorption of inhibitors with residual lignin due to the formation of HMF, furfural and acetic acid. pH was adjusted to 9 and liquor was filtered to separate crystallized lignin. Amorphous lignin was then added at a ratio of 1/100 (lignin to hydrolyzed liquor). Liquor pH was adjusted to 4 and filtered in order to remove the lignin and salt resulting from neutralization.

2.5. Fermentation

Alcoholic fermentation was performed in hydrolyzed liquor at 160 °C with 105 °C prehydrolysis. The pH was adjusted to 4 by adding concentrated NaOH. The microorganism used in study was *S. cerevisiae*, commercial type fresh biological yeast, with moisture content of 75% in a wet base. The yeast *S. cerevisiae* was added in the concentration of 30 g/L in relation to liquor.

Nutrients were added to the fermentation liquor: 1 g/L of ammonium sulfate and 0.5 g/L of potassium phosphate. The medium to be fermented was under agitation of 150 rpm at temperature of 28 °C for 24 h (shaker flask fermentation).

2.6. Contents of sugars, ethanol and aldehydes

Contents of sugars, ethanol and aldehydes (HMF and Furfural) were established by High Performance Liquid Chromotography (HPLC), with a ProStar 210 (Varian) pump; Manual injector with a 20 μL loop; ProStar 356 (Varian) refractive index detector and 284 nm UV/visible (aldehydes); Hi-Plex H (300 mm × 7.7 mm; Varian) stainless steel analytical column. Operating conditions were as follows: Column temperature 40 °C; Mobile phase: MilliQ water with a flow of 0.6 mL/min; Analysis time: 15 min and 60 min for sugar and aldehyde contents, respectively. Internal standard sugar solutions: glucose, xylose and arabinose (Sigma 99.99% HPLC grade), congeners 5-hydroxymethylfurfural – HMF (Aldrich 99.98%) and furfural (Vetec 99.9 UV/HPLC) were used to quantify liquor components.

2.7. Thermal characterization

Thermogravimetric curves (TG) and Differential Thermal Analysis (DTA) were obtained simultaneous in Shimadzu Thermal Analyzer, using synthetic air atmosphere, mass of 10 mg, heating rate of 10 °C/min and interval of temperature of 30–600 °C.

2.8. X-ray diffraction

XRD analyses were conducted in a Shimadzu XRD 600 diffractometer, with Cu Kα radiation source, tension of 40 kV and 30 mA current. Speed applied was 2 min, with a scanning range of $2\theta = 10–50°$ [17].

3. Results and discussion

3.1. Chemical composition of sisal

The chemical composition of sisal indicated moisture, ash, extractive, lignin, hemicellulose, holocellulose and cellulose contents (Table 1).

Values of cellulose and Klason lignin found in the chemical composition of sisal (fiber, leaf and bagasse) were similar to those recorded in the literature. However, ash content was higher, which may indicate the presence of impurities due to the type of defibration. Moreover, ashes depend on the region and soil type. Extractive content was lower than reported values because of the solvent used [17,18].

The results showed that the sisal fiber had the higher cellulose and lowest lignin content compared to oil palm, rubberwood, mixed hardwood. The amount of cellulose signified the potential convertible amount of glucose in the raw materials. Lignin as a structural support for the plant contains no sugars and encloses the cellulose molecules [19]. Thus, higher lignin content makes the hydrolysis step more difficult [4].

The content of cellulose (Tables 1 and 2) present in the sisal shows a lignocellulosic material which is very promising in the acid and enzymatic hydrolysis, for utilization of hydrolyzed liquor to ethanol production.

3.2. Thermal characterization

Thermogravimetric curves (TG/DTG) of sisal fiber (Fig. 2) show three steps of mass loss and ash content of 2.9%. The first step, attributed to dehydration, occurred at 18–95 °C, displaying 11.01% mass loss. The second step, attributed to decomposition of hemicellulose and cellulose, occurred in the interval of temperature of 173–362 °C, presented 57.4% mass loss. The third step, associated with cellulose and lignin decomposition, occurred in the interval of temperature from 362 to 483 °C, with mass loss of 28.69%.

Corradini et al. (2009) in a study of thermal stability of cotton fibers, mention that the hemicellulose degraded between 240 and 310 °C, the cellulose in the temperature range from 310 to

Table 1
Chemical composition of sisal.

Component	Fiber content (%)	Leaf content (%)	Bagasse content (%)
Cellulose	52.8	32.2	27.6
Hemicellulose	19.3	17.4	20.6
Holocellulose	72.1	49.6	48.2
Lignin	13.5	21.3	22.6
Extractive	5.8	7.5	8.5
Moisture	5.7	3.8	3.8
Ash	2.1	8.0	5.7

Table 2
Cellulose content.

Cellulose	Fiber content (%)	Leaf content (%)	Bagasse content (%)
Alpha cellulose	48.8	28.0	26.3
Beta cellulose	4.0	4.2	1.3

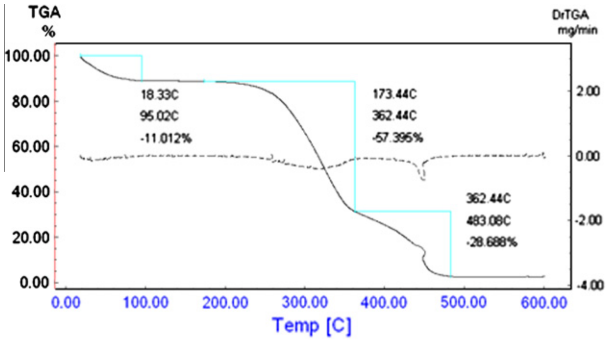

Fig. 2. TG/DTG curves of sisal fiber.

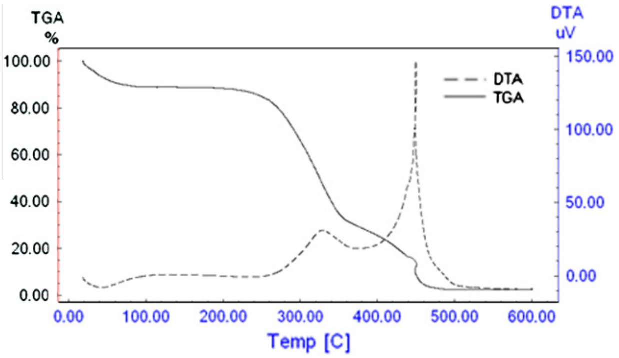

Fig. 3. TG/DTA curves of sisal fiber.

360 °C, while the lignin between 200 and 550 °C. Also say that in many cases is not possible the different processes of degradation of the components of lignocellulosic fibers, due to the reactions are complex and overlap in the range from 220 to 360 °C [20].

Kim et al. (2005) investigated the addition of lignocellulosic components to polymers given that the decomposition of cellulose occurs at temperatures below 350 °C by breaking the glycosidic bonds between carbons 1 and 4 of its monomers. They pointed out that above 400 °C is the breaking of C–O bonds of cellulose and volatile components such as CO and CH_4. On lignin, the researchers indicated higher thermal stability related to its high degree of condensation, leaving as residue, mainly phenolic compounds [21].

The DTA curve of sisal fiber presented three transitions; the transition first was endothermic, related to dehydration in the temperature interval of 25–100 °C. The transition second was exothermic, associated the cellulose and hemicellulose decomposition in the temperature interval of 260–375 °C. The transition third was exothermic, attributed to lignin decomposition in the temperature interval of 375–600 °C (Fig. 3).

The TG/DTG curves of prehydrolysis residue at 90 and 105 °C (Figs. 4 and 5) presented different profiles. The prehydrolysis residue at 105 °C showed lower stability than that prehydrolysis residue at 90 °C, which was attributed to further degradation of the hemicellulose [22].

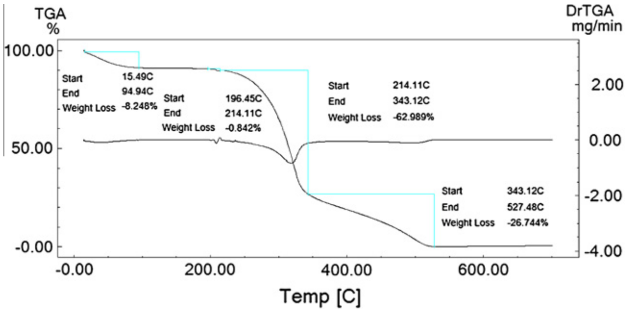

Fig. 4. TG/DTG curves of prehydrolysis residue at 90 °C.

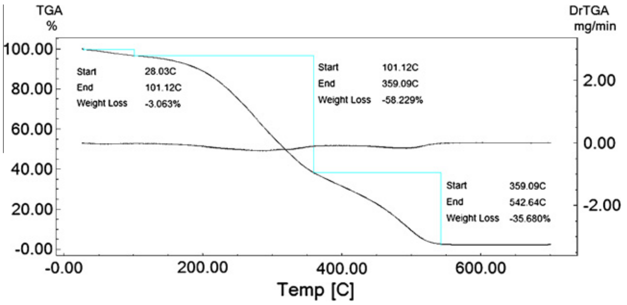

Fig. 5. TG/DTG curves of prehydrolysis residue at 105 °C.

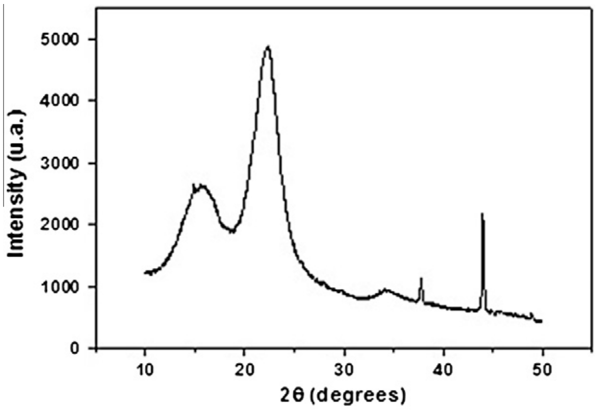

Fig. 6. Diffractogram of sisal fiber.

3.3. X-ray diffraction

The diffractogram for sisal fiber (Fig. 6) shows peaks representing the crystal planes characteristic of lignocellulosic materials: 15° (plane 101), 23° (plane 002) and 34° (plane 040). Primary components of sisal fiber are lignin, cellulose and hemicellulose. Lignin and hemicellulose are amorphous macromolecules and cellulose molecules are randomly distributed, with a tendency to form intra- and intermolecular hydrogen bonds.

3.4. Monitoring of acid hydrolysis of sisal fiber

The monitoring of hydrolysis of sisal fiber was performed at 160 °C to evaluate the best time of extraction of glucose. It was found that the best extraction of glucose occurs at the time of 1 h (Fig. 7).

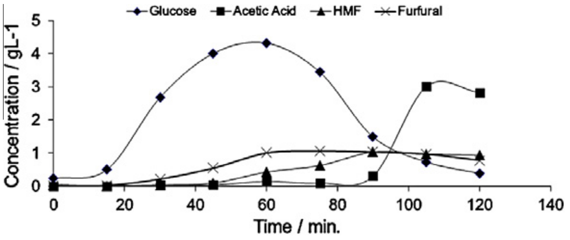

Fig. 7. Monitoring of acid hydrolysis.

The concentration of glucose in the hydrolysis liquor has a higher value at the time of 1 h, after this time there is a reduction of this concentration. There is formation of fermentation inhibitors, such as acetic acid, furfural and HMF (Fig. 7). Fig. 7 also shows that reaction time that the binomial time and temperature affect the formation of inhibitors and hence reduction of glucose and xylose in the acid hydrolysis liquor. Vancov and McIntosh (2012) evident a slight decline in xylose yield (indicative of degradation) when changed to pretreatment temperature from 30 min to 90 min [23].

3.5. Acid hydrolysis

Prehydrolysis was carried out at 90 °C and hydrolysis was performed at 160 °C (Tables 3 and 4). In this case it was verified the need to increase the concentration of glucose (hexose) in the hydrolyzed liquor, increasing the concentration of acid in the process of hydrolysis, 10%, 20% or 30%.

The advantages of the use of lignocellulosic materials as feedstock, such as sisal, are abundance in nature and the fact that there is no competition with food production [24,25].

Prehydrolysis at 90 °C was not as efficient, since it did remove little xylose. Then the prehydrolysis was performed at 105 °C (Tables 5 and 6). The greatest amount of pentoses was removed in the prehydrolysis at 105 °C compared to 90 °C, Vancov and McIntosh (2012) reported similar observations [23]. The temperature higher leads to an increased release of sugars (pentoses), probably occurs the breakage of xylose into furfural (inhibitor). Pentoses are not inhibitory but recalcitrant to fermentation into ethanol by conventional *S. cerevisiae*.

It has been known that xylose comes from the hydrolysis of hemicellulose and the pretreatment can further break xylose down to form furfural. Similarly, glucose stems mainly from cellulose hydrolysis and it can be further converted to HMF [26].

In the sisal fiber must be performed acid prehydrolysis at 105 °C, discarding the liquor of this process and then to carry out acid hydrolysis of the fiber remaining at 160 °C. Main product formed in acid hydrolysis at 160 °C was glucose, which can be metabolized by *S. cerevisiae* through alcoholic fermentation producing ethanol. For this reason, only the liquor treatment with hydrolysis of 160 °C and 105 °C prehydrolysis was used to study primary alcoholic fermentation process.

3.6. Fermentation

A preliminary study was conducted to verify the fermentation process of hydrolyzed liquor, without an optimization study of alcoholic fermentation.

Table 3
Products of acid prehydrolysis at 90 °C.

Sugar	Concentration of sugars in the liquor (g/L)
Glucose	0.4
Xylose	1.1
Arabinose	0.4

Table 4
Products of acid hydrolysis at 160 °C.

Components	Concentration in the liquor (g/L)
Glucose	5.8
Xylose	0.2
Arabinose	–
Acetic acid	2.1
Hydroxymethylfurfural	0.2
Furfural	3.4

Table 5
Products of acid prehydrolysis at 105 °C.

Sugar	Concentration of sugars in the liquor (g/L)
Glucose	0.7
Xylose	12.4
Arabinose	4.5

Table 6
Products of acid hydrolysis at 160 °C.

Components	Concentration in the liquor (g/L)
Glucose	9.3
Xylose	–
Arabinose	–
Acetic acid	–
Hydroxymethylfurfural	0.4
Furfural	2.3

Table 7
Products of fermentation.

Components	Fermentation (g/L)
Glucose	–
Xylose	–
Ethanol	4.4

Alcoholic fermentation was performed with hydrolyzed liquor at 160 °C with 105 °C prehydrolysis. Ethanol was produced with 92%, 0.47 g/g of ethanol yield conversion of initial total reducing sugars (Table 7).

Concentrations of furfural, HMF and acetic acid in the hydrolyzed liquor below 0.8, 2.0 and 2.0 g/L, respectively are not toxic to the microorganism during fermentation. The fermentation inhibitors (HMF, furfural and acetic acid) present in concentrations liquor hydrolyzate, adsorption was performed, Table 6. When the concentrations of the inhibitors are high, it is recommended the detoxification of liquor before the fermentation process, as there are strong inhibition of yeast that significantly reduces yield of alcoholic fermentation [27].

4. Conclusions

Prehydrolysis at 105 °C was effective, given that it removed a significant portion of xylose and arabinose. The main product formed by acid hydrolysis at 160 °C and prehydrolysis at 105 °C was glucose. In these conditions ethanol was produced with 92% conversion of initial total reducing sugars (optimal conversion of glucose to ethanol).

Sisal is a lignocellulosic material that demonstrates technical feasibility for ethanol production.

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